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(FILE 'HOME' ENTERED AT 08:11:51 ON 18 NOV 2004)

FILE 'MEDLINE, CAPLUS, BIOSIS' ENTERED AT 08:12:20 ON 18 NOV 2004

E (BEACHY, P)/AU
L1 246 S ((BEACHY P?) OR (BEACHY, P?))/AU
E L1
L2 121 DUP REM L1 (125 DUPLICATES REMOVED)
L3 1 S L2 AND CELL(W)ASSAY

FILE 'STNGUIDE' ENTERED AT 08:18:59 ON 18 NOV 2004

FILE 'MEDLINE, CAPLUS, BIOSIS' ENTERED AT 08:20:29 ON 18 NOV 2004

L4 98 S EXPRESS# AND LIBRARY AND HOST WITH CELL
L5 1 S L4 AND GPCR
L6 26913 S LIBRARY AND (ASSAY OR SCREEN)
L7 126 S L6 AND GPCR
L8 3937 S EXPRESS# AND LIBRARY
L9 0 S L7 AND EXPRESS# WITH LIBRARY
L10 4 S L7 AND L8
L11 4 DUP REM L10 (0 DUPLICATES REMOVED)

L10 ANSWER 1 OF 4 MEDLINE on STN

TI Cloning and characterization of a human orphan family C G-protein coupled receptor GPRC5D.

AB Recently three orphan G-protein coupled receptors, RAIG1, GPRC5B and GPRC5C, with homology to members of family C (metabotropic glutamate receptor-like) have been identified. Using the protein sequences of these receptors as queries we identified overlapping expressed sequence tags which were predicted to encode an additional subtype. The full length coding regions of mouse mGprc5d and human GPRC5D were cloned and shown to contain predicted open reading frames of 300 and 345 amino acids, respectively. GPRC5D has seven putative transmembrane segments and is expressed in the cell membrane. The four human receptor subtypes, which we assign to group 5 of family C **GPCRs**, show 31-42% amino acid sequence identity to each other and 20-25% sequence identity to the transmembrane domains of metabotropic glutamate receptor subtypes 2 and 3 and other family C members. In contrast to the remaining family C members, the group 5 receptors have short amino terminal domains of some 30-50 amino acids. GPRC5D was shown to be clustered with RAIG1 on chromosome 12p13.3 and like RAIG1 and GPRC5B to consist of three exons, the first exon being the largest containing all seven transmembrane segments. GPRC5D mRNA is widely expressed in the peripheral system but all four receptors show distinct expression patterns. Interestingly, mRNA levels of all four group 5 receptors were found in medium to high levels in the kidney, pancreas and prostate and in low to medium levels in the colon and the small intestine, whereas other organs only **express** a subset of the genes. In an attempt to delineate the signal transduction pathway(s) of the orphan receptors, a series of chimeric receptors containing the amino terminal domain of the calcium sensing receptor or metabotropic glutamate receptor subtype 1, and the seven transmembrane domain of the orphan receptors were constructed and tested in binding and functional **assays**.

PY 2001

SO Biochimica et biophysica acta, (2001 Apr 16) 1518 (3) 237-48.
Journal code: 0217513. ISSN: 0006-3002.

L10 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

TI Protein and cDNA sequences of human and rat mammalian galanin receptor GALR2

AB This invention provides the protein and cDNA sequences of human and rat galanin receptors GALR2. The invention also provides methods, vectors and host cells for expressing galanin receptor protein GALR2. A galanin receptor was isolated from a rat hypothalamic cDNA **library** and characterized in heterologous expression systems by galanin binding **assays** and receptor signaling **assays**. Like most **GPCRs**, the GALR2 receptor contains consensus sequences for N-linked glycosylation in the N-terminus (positions 2 and 11) as well as the predicted extracellular loop between TMs IV and V. The GALR2 receptor contains two highly conserved cysteine residues in the first two extracellular loops that are believed to form a disulfide bond stabilizing the functional protein structure. GALR2 shows five potential phosphorylation sites for protein kinase C in positions 138, 210, 227, 319, and 364, and two cAMP- and cGMP-dependent protein kinase phosphorylation sites in positions 232 and 316. GALR2 mRNA was observed in brain, lung, heart, spleen, and kidney, with lighter bands in skeletal muscle, liver, and testis. In LM(tk-) cells stably expressing the rat GALR2 receptor cDNA, porcine galanin (1-29) was found to inhibit the formation of cAMP induced by 10 μ M forskolin. The phosphoinositide response mediated by the rat GALR2 receptor suggested that this receptor can also couple to the intracellular calcium mobilization and diacylglycerol pathway. The pharmacol. properties identified a new receptor subtype named GALR2. A human homolog the the rat GALR2 receptor

was also cloned. Human hippocampus and human hypothalamus were each shown to **express** the intronless form of GALR2. A full-length, intronless human GALR2 PCR product was amplified from human hippocampus, but was found to contain a single point mutation downstream from the intron splice site. The invention claims GALR2 receptor subtype-selective agonists and antagonists as therapeutic agents.

PY 2004
1999
1997
1997
2003

SO U.S., 72 pp., Cont.-in-part of U.S. Ser. No. 721,837, abandoned.
CODEN: USXXAM

L10 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

TI Yeast cells having mutations in CAV1 and expressing a heterologous G protein coupled receptor, and uses therefor in drug screening **assays**

AB The invention is based on the identification of yeast protein (Cav1) homologous to mammalian caveolin, and construction of yeast cells that have a mutation that renders the yeast Cav1 protein nonfunctional. The invention provides isolated yeast cells which comprise a mutation in an endogenous yeast CAV1 gene, which exhibit increased signaling via the pheromone response pathway. In a preferred embodiment, the cav1 mutant yeast cells of the invention also **express** a heterologous G protein coupled receptor (GPCR) that functionally couples to the pheromone response pathway. The instant yeast cells display enhanced sensitivity to ligand induced stimulation of heterologous **GPCRs** and, therefore, show improved properties in drug (GPCR modulator) screening **assays**.

PY 2001
SO U.S., 31 pp.
CODEN: USXXAM

L10 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2004 ACS on STN

TI Cloning of a cDNA sequence encoding ligand (SExCkine) of human for a G protein-coupled receptor by expression cloning method

AB The invention relates to a method for isolating a nucleic acid (e.g., genomic DNA, cDNA) encoding a ligand for a G protein-coupled receptor (**GPCR**). The method comprises providing one or more primary pools of prokaryotic cells into which an expression **library** comprising exogenous nucleic acids has been inserted, and said cells having been cultured under conditions suitable to produce individual colonies and a predetd. number of said colonies having been combined into one or more primary pools of prokaryotic cells. The invention provides method of 1: expressing the expression **library** from a pool to produce a corresponding pool of proteins; 2: assaying a pool of proteins for ligand, and selecting a pool of proteins that comprises ligand; 3: providing prokaryotic cells into which the expression **library** encoding the pool of proteins selected in 2: has been inserted, and growing the cells under conditions suitable to produce individual colonies. The method further describes; 4: combining a predetd. number of colonies from 3: into a secondary pool of cells, wherein the secondary pool comprises fewer colonies than said primary pool of prokaryotic cells; 5: repeating 1 through 4 until a pool of cells containing an exogenous nucleic acid encoding ligand is isolated, said pool of cells comprising cells obtained from a single colony; and 6: recovering the nucleic acid encoding said ligand. In another embodiment, the expression **library** is expressed in vitro. The pools of expressed proteins can be assayed for ligand in a direct or indirect (e.g., competitive) receptor binding **assay** or in a functional **assay** using cells that **express** **GPCR**. In one embodiment, pools of proteins are assayed for ligand

in a chemotaxis **assay** using recombinant cells which
express GPCR.

PY 2001

SO PCT Int. Appl., 42 pp.

CODEN: PIXXD2